# Cryptic dispersal in non-acidic environments from Turkey of Cyanidiophytina (Rhodophyta)

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**Abstract**

Cyanidiophytina are a group of polyextremophilic red algae with a worldwide, but discontinuous colonization. They are restricted to widely dispersed hot springs, geothermal habitats, and also some human-altered environments. Cyanidiophytina are predominant where pH is prohibitive for the majority of eukaryotes (pH 0.5-3). Turkey is characterized by areas rich in volcanic activity separated by non-volcanic areas. Here we show that Cyanidiophycean populations are present in thermal baths located around Turkey on neutral/alkaline soils. All known genera and species within Cyanidiophytina were detected in Turkey, including *Galdieria phlegrea*, recorded up to now only in Italian Phlegrean Fields. By phylogenetic analyses, Turkish *G. sulphuraria* strains are monophyletic with Italian and Icelandic strains, and with Russian *G. daedala* strain*s*. *G. maxima* from Turkey clustered with Icelandic, Kamchatka, and Japanese populations. The discovery of Cyanidiophytina in non-acidic Turkish soils raises new questions about the ecological boundaries of these extremophilic algae. This aids in the understanding of the dispersal abilities and distribution patterns of this ecologically and evolutionarily interesting group of algae.

**Keywords:** Extremophiles, Cyanidophytina, Phylogeny, Population structure, rbcL, Biodiversity

## Introduction

Cyanidiophytina (Rhodophyta) are a group of red unicellular algae highly adapted to the environmental extremes offered by volcanic regions. These environments often support temperatures above 50 ºC and have high sulfuric acid concentrations that results in acidic pH levels prohibitive for most eukaryotes (Albertano et al. 2000; Brock 1978; Pinto et al. 2003; Pinto et al. 2007; Cennamo et al. 2017). The interest in global biodiversity and distribution patterns of thermoacidophilic Cyanidiophiceaen populations led to numerous explorations of volcanic regions both in and outside of Europe, such as Italy, Iceland, USA, New Zealand, and Japan. In this, molecular approaches were successfully used to assess the level of biodiversity in this group (Ciniglia et al. 2004; Yoon et al. 2004, 2006; Toplin et al. 2008, Ciniglia et al. 2014). This provided a hypothesis of the origin and dispersal routes of *Galdieria maxima* and *G. sulphuraria* in populations from Iceland and northeastern Asia. Cyanidiophytina mobility is still poorly understood.

A novel estimate of species richness of Cyanidiophyceae has recently come from the analysis of thermoacidophilic communities from aquatic and non-aquatic volcanic sites in Taiwan (Hsieh et al. 2015). The habitats so far explored, in search of polyextremophilic algae, have usually been characterized by strong acidity, as pH range is considered a greater constraint on the growth of Cyanidiophytina than temperature range. Thus, many explorations have focused in acidic geothermal areas (Brock 1978, Toplin et al. 2008, Hsieh et al. 2015). Currently, the genus *Cyanidium* encompasses two main species. These are *C. caldarium* (Tilden) Geitler, a polyextremophilic alga adapted to acidic and hot springs and fumaroles, usually rich in heavy metals, and *C. chilense*, a hypogean, neutrophilic (pH around 7.0) and mesophilic (20-25 °C) alga discovered in several caves worldwide (Schwabe, 1936; Friedmann, 1964; Skuja, 1970; LeClerc et al., 1983; Azua-Bustos et al., 2009; Darienko et al., 2010; Del Rosal et al., 2015; Cennamo et al., 2012; Ciniglia et al. 2017). The phylogenetically distinct thermoacidophilic *C. caldarium* and the neutrophilic and mesophilic *C. chilense* are clearly separated on the basis of both molecular and ecophysiological characters (Ciniglia et al., 2004). These findings suggest that other Cyanidiophytina could have a much wider distribution than those considered so far. This prompted us to search for alternative ecological niches, such as non-acidic environments.

In this study, we report on our new explorations of seven thermal baths located in Turkey and report the presence of Cyanidiophycean populations on neutral/alkaline soils. Anatolian volcanism is a consequence of convergence occurring between Afro-Arabian and Eurasian plates and it can be considered as a bridge between the geothermal areas of Europe

and Asia. This zone is characterized by deposits of andesitic and rhyolitic lava, alternating with black and clastic sedimentary rocks, resulting from the solidification of mud mixed with water (Pearce et al., 1990). Although Turkey is still geologically active, intense volcanic activity has not been recorded for a number of years; Turkish volcanism varies from mildy alkaline volcanoes, such as Nemrut, to calc-alkaline/alkaline volcanoes, such as Ararat and Tendurek (Pearce et al. 1990).

The chemical composition of rocks collected in our sampling areas was determined by X-ray diffraction. Next a culture-dependent approach combined with rbcL gene sequencing was employed to characterize the phylogenetic positioning of algal diversity of the Cyanidiophycean populations we isolated from Turkey. We also added all of the available rbcL gene sequences from a wide geographic range, to refine the population structure and molecular variance. Then we explored the geographical distribution of global genetic variation in different species and genera of Cyanidia.

## MATERIALS AND METHODS

### *X-ray diffraction (XRD)*

XRD was performed on the mineralogical phases of substratum inorganic components occurring in the algal biofilms. XRD patterns were collected in the 3–90° 2θ range, according to the step scanning procedure with Co radiation on a Miniflex Diffractometer (Rigaku, Japan). The tube operated at 30 kV and 15 mA, and the counting time was 3600 s. The identification of mineralogical phases was performed with a search/match on the Joint Committee on Powder Diffraction Standards.

### *Sample collection, isolation and cultivation*

Environmental samples were collected from seven Turkish thermal stations located in the south eastern, north eastern, and south western peninsula: 1) Cermik-Diyarbakir, 2) Biloris-Siirt; 3) Güçlükonak-Şirnak; 4) Nemrut crater lake-Bitlis; 5) Agri-Diyadin; 6) Kula-Manisa; 7) Germencik-Aydin (Fig. S1). For each station, samples were collected where algae were present either superficially or covered by crystals, crumbly soil, and mud layers, respectively (Fig. 1). The samples were collected from different microenvironments, such as the surface of the crystals, around the granules of crumbly soil and between the layers of mud (Fig. 1). Temperatures were measured with a digital thermometer (Field Environmental Instruments, Pittsburgh, Pennsylvania, USA). pH was measured with a portable pH meter (Hanna Instruments, Padova, Italy) and with pH strips (Macherey Nagel Bethelem, USA).

Sampling location, coordinates, pH, temperature, and habitat for each sampling site are summarized in Table 1. All samples were collected by scraping the mineral substratum and these were stored in sterile tubes. In order to obtain monoclonal cultures of each sample, serial dilutions were performed in a specific medium for Cyanidiophytina (Allen's medium, pH 1.5, Allen & Stanier 1968); multi-well plates were used for the isolations. Maximum dilution enrichments were also streak-plated onto Allen's medium supplemented with agar. Single colonies were chosen from each plate and suspended in liquid Allen's medium. Cultures in both tubes and plates were grown at 37°C under continuous fluorescent light. All isolates were numbered and stored in the Algal Culture Collection of University Federico II of Naples (ACUF, www.acuf.net). Cultures are available upon request to the authors.

Algal samples were inspected using a light microscope (Nikon Eclipse E800 equipped with Nomarski interference), in order to visualize strains grown in Allen's medium.

115 ***DNA extraction, gene amplification and sequencing***

116 For DNA extraction, algal cells were suspended in a specific buffer (DNeasy Plant
117 Mini Kit, Qiagen, Santa Clarita, California, USA) and ground with glass beads using a Mini-
118 BeadBeater (BioSpec, Bartlesville, Oklahoma, USA) operated at 13,000 revolutions per min
119 for 5 min. Total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Santa
120 Clarita, California, USA). Four degenerate primers were used to amplify the rbcL gene from
121 isolated samples (Ciniglia et al. 2004). The resultant products were purified with the
122 QIAquick PCR purification kit (Qiagen) and used for direct sequencing using the BigDyeTM
123 Terminator Cycle Sequencing Kit 3.1 (PE-Applied Biosystems, Norwalk, Connecticut, USA)
124 and an ABI-3500 XL at the Microgem Laboratory (Naples, Italy). Forward and reverse
125 electropherograms were assembled and edited using the program Chromas Lite v.2.1
126 (www.technelsium.com.au/chromas.html).
127

128 ***Phylogenetic analyses***

129 A total of 81 new rbcL sequences were obtained in this present study from our Turkish
130 samples, and these were integrated with the 255 available rbcL sequences available at
131 GenBank (Table S1). All sequences were aligned with published sequence data (Ciniglia et al.
132 2004, Toplin et al. 2008, Skorupa et al. 2013, Ciniglia et al., 2014, Hsieh et al. 2015), using
133 BioEdit Sequence Alignment Editor (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). No
134 gaps or indels have been incorporated in the alignment. Newly determined sequences are all
135 available on NCBI GenBank (Table S1). Maximum likelihood (ML) phylogenetic analysis of
136 rbcL was performed using the GTR + Γ + I model implemented in RAxML software
137 (Stamatakis 2008). Statistical support for each branch was obtained from 1,000 bootstrap
138 replications using the same substitution model and RAxML program settings. Bayesian
139 analyses (BA) were performed for combined and individual datasets with MrBayes v.3.1.1
140 (Ronquist and Huelsenbeck 2003) using the Metropolis-coupled Markov chain Monte Carlo
141 (MC3) with the GTR + Γ + I model. For each matrix, one million generations of two
142 independent runs were performed with sampling trees generated every 100 generations. The
143 burn-in period was identified graphically by tracking the likelihoods at each generation to
144 determine whether they reached a plateau. Seven red algal taxa belonging to Bangiophyceae
145 and Stylonematophyceae were chosen as outgroup taxa, being the closest relatives to
146 Cyanidiophytina.

147 An estimate of genetic diversity was carried out using DNASP v.5.10.01 (Librado &
148 Rozas 2009). For each population, the following statistics were computed: haplotype (h) (Nei
149 1987) and nucleotide diversities ($\pi$) (Nei 1987), with standard deviation. Population
150 expansion, assessed by neutrality test (Tajima 1989, Fu&Li 1993) and mean number of
151 pairwise differences (symbol) (Tajima, 1983).
152 To assess population differentiation, pairwise $F_{st}$ values were calculated as the
153 pairwise genetic differentiation (pairwise $F_{st}$ statistics) in ARLEQUIN version 3.5.2.2
154 (Excoffier and Lischer 2010) based on 50,000 permutations ($P < 0.05$). The isolation-by-
155 distance was tested using a Pearson correlation in R, testing for a positive correlation between
156 pairwise geographic distance (in km) and Fst average pairwise differences.

## RESULTS

Soil and rock samples at the Anatolian volcanism region were surveyed in search of Cyanidiophytina species. Table 1 shows the location of the sampling sites, temperature and pH, along with the type of substratum for each sampling station. In all of the examined samples, quartz and potassium feldspars were the main minerals found, followed by calcyte (Kula Manisa and Germencik), pyroxenes and dolomites (Agri-Dyadin, Cermik-Dyiarbakir and Biloris-Sirt), and gypsum (Gucklukonak-Sirnak). These Turkish sites had mostly neutral pHs (Table 1). Despite this, all collected samples had Cyanidiophycean. We were successful in isolating cultures at all sites using Allen's medium at pH 1.5. Cyanidiophycean cultures grew abundantly, suggesting that although adapted to neutral soil, these microalgae were acid tolerant. The same medium was used to obtain single colonies, and axenic cultures were deposited at the Algal Collection of University Federico II (ACUF, www.acuf.net).

The identification of different genera and species in the Cyanidiophytina has previously been difficult, as there are few unequivocal morphological features to distinguish between them, and furthermore, there is homoplasy between some lineages. Thus, in order to identify the algal species been studied molecular tools were used. For this we first generated 491 base pairs of rbcL sequence for the different isolates. These were aligned, including the 81 new Turkish cyanidiophycean isolates, (Table S1), and the existing 168 cyanidiophycean rbcL sequences available from GenBank. These strains originated from Japan, Iceland, Italy, Kuril Islands, Kamchatka, USA, New Zealand, and seven outgroup taxa. rbcL phylogeny identified five cyanidiophycean taxa from Turkey: *Galdieria sulphuraria, Galdieria maxima, Galdieria phlegrea, Cyanidium caldarium*, and *Cyanidioschyzon merolae* (Fig. 2). The inferred RAxML tree based on rbcL dataset showed several well-supported sublineages within *G. sulphuraria* and *G. maxima* clades. *G. sulphuraria* included at least five sublineages, including on defined by a New Zealand population (Fig. 2, subclade S1) and another with a USA population (Fig. 2, subclade S2). Accessions nested in an independent lineage, separable as two well-supported subclades (posterior probability/bootstrap: New Zealand subclade, 1/97; USA subclade, 1/98). We noted that 10 Turkish specimens grouped within *G. sulphuraria* and this was in two different subclades, 7 nesting with the Italian strains (Fig. 2, subclade S3; posterior probability/bootstrap 1/100), and 3 with the Icelandic strains along with the Russian *G. daedala* strain (Fig. 2, subclade S5; posterior probability/bootstrap 1/69). The sequences from Taiwan clustered with *G. partita* from Russia. Together the relations were clearly resolved with high statistical confidence.

The *G. maxima* assemblage included four subgroups reported in M1 to M4. Turkish specimens of *G. maxima* (n=40) clustered in two well-supported, different subclades, 13 of which clustered with Icelandic specimens (Fig. 2, subclade M1), 27 nesting with conspecific strains from Japan, Taiwan, and the Russian *G. maxima* authentic strain (Fig. 1, subclade M2). rbcL sequences from Taiwan and Japan both grouped into two subclades M2 and M3. *G. maxima* sequences from New Zealand did not group with any of conspecific strains collected from other locations, as in *G. sulphuraria* (Fig. 2, subclade M4).

The *G. phlegrea* clade was formed by Turkish (n=26) and Italian (n=8) isolates. This was strongly supported by high posterior probability/bootstrap values of 0.94/97%. *C. caldarium* from Turkey (n=3) were closely related to all other isolates with 100% bootstrap value.

Only two Turkish isolates were found to be closely related to *C. merolae*. The low level of intraspecific variation recorded in *C. merolae* did not generate any subclusterization associated to geographic populations. Our phylogenetic tree conformed to previously reported monophyly of Cyanidiophyceae (posterior probability, 1; ML LogDet bootstrap = 100%) (Fig. 2) (Ciniglia *et al.* 2004). However, by adding the rbcL sequences from the new Turkish isolates, at least six lineages within the class were indicated by the high bootstrap values, instead of the previously reported four lineages (Ciniglia *et al.* 2004; Ciniglia *et al.*, 2014). These six independent lineages were grouped in different monophyletic clades (Fig. 1), namely: 1) *C. merolae* (posterior probability 1/bootstrap, 99%); 2) *G. maxima* (1/99), sharing a common ancestor with *C. merolae*, but with strong evidence of molecular divergence between them; 3) the mesophilic lineage of *C. chilense* (1/100; Ciniglia *et al.*, 2017)*;* 4) *C. caldarium* (1/100), clearly phylogenetically divergent from the mesophilic *C. chilense* (Yang *et al.*, 2016); 5) *G. sulphuraria* (1/100) and 6) *G. phlegrea* (0.94/97), as sister clades (1/ 100).

***Genetic diversity and population differentiation***

Next an analysis of genetic diversity within and between populations of Cyanidiophyceae was performed by using DNAsp, which provides an estimate of the extent of genetic variation between individuals belonging to the same geographic population and between different populations. Results are listed in Table 2. We excluded *C. caldarium* from the analysis because of the low number of haplotypes and their restricted geographic distribution. A total of 159 haplotypes were recovered from 459 individuals analyzed and 149 (95.5%) of the haplotypes were private, *i.e.* unique to a single locality. The highest values of

224 average sequence divergences were recorded for *G. sulphuraria* (K=19.47), and *G. maxi*ma
225 (K=17.37), with a high level of haplotype diversity, as well (*G. sulphuraria*, hd, 0.83±0.028;
226 *G. maxima*, hd, 0.956±0.006).
227 In *G. sulphuraria*, the analysis of genetic diversity was performed on 136 partial
228 sequences of rbcL with 80 polymorphic sites and 33 different haplotypes (only two
229 haplotypes were shared by Italy and Turkey and by Taiwan and Russia). The highest levels of
230 haplotype diversity were found in the samples from New Zealand (hd = 0.867), Italy, and
231 Taiwan (hd = 0.724 and 0.732). An average value of haplotype diversity was recorded in
232 Turkey (hd = 0.600), despite the degree of nucleotide diversity higher than any other
233 population ($\pi$ = 0.0375). Iceland exhibited comparatively lower values of these indices (hd =
234 0.224; $\pi$= 0.0006).
235 Genetic distance was represented as Fst for each pairwise combination of populations,
236 based on rbcL marker. The value of inter-populational pairwise genetic differentiation, Fst (5
237 populations of *G. sulphuraria* analyzed: USA, Italy, Turkey, New Zealand, and Iceland) was
238 significantly high (0.7788, $P<0.05$). Fst ranges from 0 to 1; Fst of 0 indicates panmixy with
239 high interbreeding between populations, while a value of Fst of 1 means that the populations
240 are fixed and do not interbreed. When considering the genetic differentiation between two
241 populations, Fst values ranged from low (0.14) to high (0.97) (Table 3). The lowest level of
242 genetic differentiation was recorded between Turkey and Italy, which were also the closest
243 populations geographically (1950 km). However, high genetic divergences were found
244 between the furthest and the closest *G. sulphuraria* populations, such as Taiwan and USA
245 (0.97, 12254km), USA and Iceland (0.91, 5719km), Italy and USA (0.85, 8622km), New
246 Zealand and Iceland (0.844, 17215km), Italy and Iceland (0.839, 3247 km), and New Zealand
247 and Italy (0.71, 18559km). We next investigated the potential for isolation by distance (IBD)
248 via statistical tests of correlations in order to weigh the contribution of geographic distance in
249 the population structure. The correlation between genetic and geographic distances based on
250 rbcL was weakly positive, but not statistically significant in *G. sulphuraria*, as shown in
251 Fig. 3 (R=0.264, P=0.333). This thus rejected an isolation-by-distance model from these data.
252 In examinations of 245 *G. maxima* partial rbcL (434bp) sequences, these contained
253 161 polymorphic sites and 100 haplotypes (Table 2). There was a high level of detected
254 diversity (hd=0.956). Haplotype and genetic diversity of rbcL in Turkish populations,
255 calculated from 40 sequences and 8 haplotypes were 0.652±0.069 (hd) and 0.02±0033 ($\pi$) in
256 43 polymorphic sites. The highest genetic diversity was found in the Taiwanese population,
257 where among 149 individuals, 80 haplotypes and 108 parsimony informative sites showed

high haplotype diversity (0.957±0.009) with low genetic polymorphism ($\pi$=0.0373±0.00067). The Japanese population was the highest in both diversities (hd=0.861±0.039; $\pi$=0.023±0.00345). This resulted from 23 sequences, 8 haplotypes, and 34 polymorphic sites. The level of haplotype and nucleotide diversity for the New Zealand population was calculated on the few sequences available (7 individuals, 4 haplotypes, hd=0.81±0.13, $\pi$=0.028±0.006). The 24 Icelandic sequences showed a lower haplotype and nucleotide diversity (hd=0.163±0.0098; $\pi$=0.00073±0.00051). In the neutrality test of *G. maxima*, Tajima D and Fu and Li were both significantly negative for the Icelandic samples (D= -1.88381; F= -2.796 Table 2). However, all samples from the other regions showed negative values of Tajima D, but without statistical significance of Tajima and Fu and Li, except for Taiwan samples showing strong significantly negative values of F (Table 2).

The inter-populational genetic differentiation, Fst calculated on 5 *G. maxima* populations (Turkey, Japan, Iceland, New Zealand, and Taiwan) was 0.55. However, the highest similarity in genetic structure calculated between two populations was accounted for the geographically closest populations Japan and Taiwan (Fst= 0.162). Low levels of genetic differentiation were also found between Turkey and Taiwan (Fst= 0.287) and Turkey and Japan (Fst= 0.257), despite the significant geographic distances between them. The highest Fst value was exhibited between Iceland and New Zealand, areas geographically far apart. A weakly positive correlation between genetic and geographic distances was detected for *G. maxima*, although it was not significant (R=0.145; P=0.763, Fig. 3).

Despite extensive sampling, current and previous molecular analysis has to date only identified 44 rbcL sequences from *C. merolae*. The majority belonged to individuals spread across the American territories, as few sequences were detected in the Turkish or Italian samples, and no sequences have yet been detected in Taiwanese samples. The analysis revealed the presence of 19 polymorphic sites, generating 19 haplotypes. The two most frequently represented were shared by the Turkish, Italian, and American samples. Genetic haplotype diversity was estimated using all of the isolates and gave results of 0.918±0.022, with a very low degree of nucleotide diversity, namely $\pi$=0.00443±0.00219 (Tajima, -1,73184; Fu and Li, -3,456). This indicates the absence of geographical population structuring. This was also shown by the low level of the overall genetic differentiation (Fst=0.05). We could not perform correlation test for *C. merolae*, as well as for *G. phlegrea* and *C. caldarium*, because of the limited number of accessions and populations available for the analysis.

## DISCUSSION

Cyanidia are the most abundant photosynthetic protists found in extremely acidic, sulfur-rich environments that are close to active volcanoes (Brock *et al.*, 1978; Ciniglia *et al.*, 2004; Skorupa *et al.*, 2013; Toplin *et al.*, 2008). Until now Cyanidia have been isolated mainly in solfataras (Italy, Iceland, Japan, New Zealand, Yellowstone National Park, and Taiwan), where the condensation of sulfur dioxide and hydrogen sulfide produces crystals of sulfur subsequently oxidized to sulfuric acid resulting in acidification.

Turkey is characterized by collision volcanism, varying from mildly alkaline volcanoes, such as Nemrut, to calc-alkaline/alkaline ones, such as Mount Ararat (Pearce et al., 1990). Residual volcanic activity in Turkey explains the presence of many geothermal spots, with neutral and sub-neutral pH values, due to the limited presence of sulfuric acid. The main minerals detected in the areas explored were quartz, feldspars, calcite, and dolomites (Table 1). Narrow and thin biofilms of Cyanidia were detected in Turkish thermal baths, mostly in hypolithic and endolithic conditions.

Most of the species isolated from Anatolia were highly acidotolerant organisms, able to survive in a wide range of pH conditions (*Galdieria maxima, Galdieria phlegrea*, and *Cyanidium caldarium* between 1 and 7, *Galdieria sulphuraria* between 1 and 5.8). However, all species and strains, regardless of the ecological features of the sampling sites, remained well suited to acido-thermal or at least acidic growth conditions. One exception is represented by *Cyanidium chilense* (=cave *Cyanidium*, Schwabe, 1936, 1942; Hoffman, 1994; Ciniglia et al., 2017), which represents a separate monophyletic lineage within Cyanidiophytina, including several strains dispersed worldwide. It appears to be limited to cave habitats where pH and temperature are not extreme, and is unable to proliferate in laboratory conditions. Cyanidiophytina are thus abundant in mesophilic areas of Turkey, but are still adapted to thrive under acido-thermal environment.

According to Doemel and Brock (1971), the occurrence of *C. caldarium* in non-thermal habitat was frequent, being recorded in aquatic habitats between 20 °C and 55 °C and on soils at temperature between 10 °C and 55-57 °C. Pinto *et al.* (1993) similarly reported the presence of *C. caldarium*, *G. sulphuraria*, and *C. merolae* in more than 100 hydrothermal sites around Italy. These were not only in acidic hot springs, but also in acidic non-thermal ones, such as the sulfur mines. Recently, Hsieh et al. (2015) identified a novel mesophilic *Cyanidium* clade from non-thermal, but acidic sites in Taiwan, thus supporting the frequent occurrence of Cyanidiophytina in geothermal environments not necessarily in high temperature conditions (Gross et al., 2002).

Lowell & Castenholtz (2013) tested the ability of several *Cyanidium* to lower the external pH from 6 to more acidic values. They confirmed that many *Cyanidium* obtained from Yellowstone, Japan, Philippines, and New Zealand hot springs could acidify their growth environment. This suggested the importance of this process as survival strategy in confined environments, such as microbial mats, interstitial soil spaces, and endolithic niches. These algae appear to harbor adaptive responses to survive the non-ideal conditions during their dispersal, helped by wind flow, air particles, or birds. Despite the limited tolerance to desiccation and the absence of resting spores for Cyanidiophytina (Gross et al. 2002), the ability to lower the pH outside the cell would render them able to survive in non-acidic environments. This could potentially serve as a connection between the thermoacidic locations as a mechanism of long-distance migration (Brock, 1978; Gross, 1999).

The molecular investigations on new cyanidiophycean isolates revealed the presence of all representatives of this class of microalgae, namely *G. sulphuraria*, *G. phlegrea*, *G. maxima, C. merolae*, and *C. caldarium* on hydrothermal soils around Turkey. The new rbcL sequences were mostly attributed to *G. phlegrea* and *G. maxima*, while *G. sulphuraria*, *C. merolae*, and *C. caldarium* sequences were rarely detected. Turkey is the first site in which all these species have been collected in one local. For example in Italy *G. maxima* has not yet been detected, while all other thermoacidophilic communities sampled to date have an incomplete number of species and strains (Toplin *et al.*, 2008; Skorupa *et al.*, 2013, Hsieh *et al.*, 2015).

Of remarkable interest is the detection of *G. phlegrea* in almost all of the sampling stations from Turkey, recorded to now only in one Italian area located within the Phlegrean Fields (Naples, Italy), adapted to relatively dry areas and to dim light (Ciniglia *et al.*, 2004; Pinto *et al.*, 2007). *G. phlegrea* possesses interesting ecophysiological traits, exhibiting maximal growth at 25 °C, which is lower than *G. sulphuraria* at 38 °C. It is known that amongst Rhodophyta, all Cyanidiophytina encountered an extensive reduction of their genome. It has been proposed that this is an adaptation strategy to stressful environmental conditions. *G. phlegrea* have regained genes through horizontal gene transfer, suggested as an ameliorative strategy for adaptation to specific environmental niches (Qiu *et al.*, 2013).

Genomic analyses revealed that *G. phlegrea* and *G. sulphuraria* belong to different taxa, since the protein divergences between them are comparable to the protein-divergence distances between humans and teleosts (Qiu *et al.*, 2013). The rbcL sequences of Turkish *G. sulphuraria* isolates showed the highest genetic variability both in terms of haplotype diversity and in nucleotide diversity, followed by Taiwanese conspecific specimens. *G.*

359 *sulphuraria* strains from Turkey clustered in two separate lineages, the former including
360 Italian isolates, the latter including Icelandic strains. This finding suggests that there have
361 been at least two separate introductions from Turkey in Western Europe; the levels of
362 interpopulational genetic differentiation suggested a dispersal ability significantly higher
363 between Turkey and Italy than between Turkey and Iceland, which would be consistent with a
364 correlation between genetic and geographic distance.
365 Ciniglia *et al.* (2014) previously hypothesized that the northeastern Asian populations
366 of *Galdieria* would be the potential donor of Icelandic *G. sulphuraria* populations, because of
367 the occurrence of the Russian species *G. daedala* within the same clade, alongside some
368 Turkish accessions. The strong monophyly among Turkey, Iceland, and Russian strains, along
369 with the highly divergent haplotypes associated with Turkish accessions, would be consistent
370 with Turkey in being a center of *G. sulphuraria* diversification and dispersal to Western
371 European sites. A similar pattern was found in the *G. maxima* clade; Turkish isolates strictly
372 grouped both with Icelandic and with Japanese and Taiwanese accessions, along with the
373 Russian haplotype *G. maxima* IPPAS P507. In the present study, the combination of high
374 haplotype and low nucleotide diversity is a signature of a rapid population expansion from a
375 small effective population size (Avise 2000); Tajima's D test and Fu's Fs tests, applied to find
376 out the population expansion, were both negative in all cases; this indicates excess of the rare
377 mutations in populations, thus supporting the hypothesis of recent population expansions
378 within Cyanidiophytina.
379 The discovery of Cyanidiophyceae in Turkey confirms the cosmopolitan distribution
380 of these algae, despite the peculiar ecological requirements that are present in discontinuous
381 and distant habitats. The worldwide distribution of extremophiles has been demonstrated also
382 for *Sulfolobus,* an archea inhabiting the geothermal sulfuric springs at T > 70 °C and strongly
383 acidic pH, isolated in several hot springs throughout Northern hemisphere (Brock et al., 1972;
384 Zuo et al., 2015). It is intriguing for these extremophiles, such as the Cyanidiophytina, to
385 understand how they can survive long-distance dispersal, through inhospitable environments,
386 without tolerating desiccation and without producing resistance spores.
387 We examined the population structure in *G. sulphuraria*, *G. maxima*, *G. phlegrea*, and
388 *C. merolae*, measuring Fst, a parameter that provides a measure of population differentiation
389 based on genetic variance between the populations. Pairwise comparisons between strains
390 grouped by region have produced different results in the Cyanidiophycean taxa. Large,
391 significant Fst values across the hydrothermal locations were recorded in *G. sulphuraria* and
392 *G. maxima* suggesting a high level of genetic differentiation, and a reduction in dispersal

393 ability of the individuals. However, in *G. maxima* low Fst values were recorded among the
394 Asiatic populations, indicating that there is at least a small level of genetic differentiation
395 between them, and a substantial level of gene flow. Perhaps this was due to the contiguity of
396 the geothermal areas, being located on the Ring of Fire. Within *C. merolae* and *G. phlegrea,*
397 Fst values were not significantly different from zero (Fst= 0.05 and 0.013, respectively),
398 indicating that populations from different geothermal springs were not genetically
399 differentiated, suggesting a frequent gene flow among the geothermal springs. *G. phlegrea*
400 populations to date have only been identified in Turkey and Italy, and it is intriguing that even
401 in *G. sulphuraria*, the lowest level of genetic differentiation was recorded between the same
402 populations. This supports the hypothesis of gene flow between Turkey and Italy. The level of
403 genetic divergence of *G. phlegrea* was much lower than that observed in *G. sulphuraria* and
404 in *G. maxima. G. phlegrea* has a restricted areal of dispersal, because of its peculiar
405 adaptation to dry habitats, such as rock fissures, chasmoendolithic and cryptoendolithic
406 environments. These habitats were very frequently encountered in Turkey, and are preferred
407 by *G. phlegrea* in spite of fumaroles and hot springs.
408 Significant levels of genetic divergence were reported for other extremophilic
409 microorganisms, such as in populations of *Sulfolobus solfataricus* where gene flow among
410 different geothermal stations is limited (Whitaker et al., 2003). However, while in *S.*
411 *solfataricus* the global population structure is mainly ascribed to isolation by distance, in
412 Cyanidiophytina, namely in *G. sulphuraria* as well as in *G. maxima*, gene flow and species
413 dispersal among populations was not found to increase with the geographic distance. This is
414 notable as there was no significant positive correlation between genetic and geographic
415 distance. For an extremophile, hot springs may be considered as island-like habitats occurring
416 as clusters in globally distant regions. For an extremophilic organism to thrive in such
417 conditions, they must adapt to drastically different conditions from the surrounding habitat
418 through which they would have to disperse (Ramette and Tiedje, 2007). As such, it would be
419 expected that geographical isolation might be an important component in the diversification
420 of microextremophiles (Papke et al., 2003), as already observed in *S. solfataricus* (Whitaker
421 et al., 2003). In stark contrast, our results suggest that for Cyanidiophyceae, their growth
422 requirements limit dispersal, but do not prevent it. The discovery of such a high number of
423 Cyanidiophycean species and strains from global explorations is helpful to better delineate
424 ecological boundaries. Moreover, the phylogenetic analyses strongly support the
425 reconstruction of the relationships between the 6 lineages recovered. For this purpose,
426 sequencing of the whole rbcL gene as well as additional markers, such as the nuclear small

427 and large subunit rDNA genes (SSU and LSU), concatenated with rbcL, should result in a
428 substantial improvement in phylogenetic resolution.

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## ACKNOWLEDGEMENTS

We are especially grateful to Dr Nurullah Akcan for his help in the exploration of the thermal areas around Turkey. We also thank Dr Rachael Oakenfull for English language editing.

## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author(s).

## AUTHOR CONTRIBUTIONS

A. Eren: conduction of experiments, analysis of results, contribution to draft writings; M. Iovinella: conduction of experiments, analysis of results, contribution to draft writings; S. J. Davis analysis of results, contribution to draft writings; D. Cioppa: isolation of strains, conduction of experiments; G. Pinto and A. Pollio: original concept, provision of resources; C. Ciniglia: original concept, provision of resources, draft editing.

**Figures and Tables legends**

**Fig. 1**. Pictures of some sampling points from the Turkish thermal areas for Cyanidiophytina. a, Cermik, Southeastern Turkey; b, c, endolithic growth of Cyanidiophytina in Germencik, Southwestern Turkey; d,e,f, Agri, Diyadin, Northeastern Turkey; g,h, Kula Manisa, Southwestern Turkey; i, Saart, Manisa, Southwestern Turkey; j, k,l, Salihli, Manisa, Southwestern Turkey.

**Fig. 2.** Consensus Bayesian tree of Cyanidiophytina based on rbcL sequences. The Bayesian posterior probability and maximum-likelihood (RAxML) bootstrap values (MLBT) are shown above the branches. Dashes indicate support values <50%.

**Fig. 3**. Correlation among genetic divergence and geographic distance. Each point represents a single pairwise comparison between seven isolated populations. Regression lines show relationships between genetic divergence and geographic distance (*G. sulphuraria*, R=0.245, P=0.333; *G. maxima*, R=0.145, P=0.763).

**Table 1.** Location, codes, habitat, pH, temperature and main minerals of sampling sites in Turkey.

**Table 2.** Statistics of rbcL haplotypes for the Turkish cyanidiophycean strains; n. sample size, v. variable sites, N. number of haplotypes, h. haplotype diversity, K. Average number of pairwise nucleotide differences, $\pi$ nucleotide diversity. (significance *: $p< 0.05$; **; $p< 0.10$).

**Table 3**. Matrix of pairwise estimates of Fst between pairs of populations of *G. sulphuraria* and *G. maxima.*

**Supplementary material**

**Fig. S1**. Map of Turkey. Names indicate the sampling sites from where Cyanidiophytina were isolated.

**Table S1.** GenBank Accession numbers for taxa included in the phylogenetic analyses.

| Location, region (coordinates) | Sampling location code | Habitat | pH | T (°C) | Minerals |
|---|---|---|---|---|---|
| Cermik, Diyarbakir Southeast Turkey (38°8'16"N, 39°28'3"E) | SET.CE | Thermal bath, on the wall inside and outside the hammam | 7 | 24.6 | Quartz, pyroxenes, dolomites |
| Biloris, Siirt Southeast Turkey (37°56'7"N, 41°56'12"E) | SET.BI | Thermal bath, on the wall, inside the hammam | 7 | 25.8 | Quartz, pyroxenes, dolomites |
| Gü.lükonak, Şirnak Southeast Turkey (37°28'10"N, 41°54'39"E) | SET.GU | Thermal bath, on the wall inside the hammam | 1 | 54 | Quartz, feldspars, gypsum |
| Nemrut crater lake East Turkey (38°37'33"N, 42°14'44"E) | CET.NE | Fumaroles | 6.7 | 32-46 | Quartz, feldspars, gypsum |
| Agri, Diyadin Northeast Turkey (39°32'26"N, 43°40'57"E) | NET.DI | Fumaroles, hot spring, hot pool, hot soil | 6.5 | 45 | Quartz, pyroxenes, dolomites |
| Kula, Manisa Southwest Turkey (38°32'45"N, 28°38'48"E) | SWT.KU | Hot soil-hot pool | 5 | 41 | Quartz, feldspars, miche, calcyte |
| Germencik, Aydin Southwest Turkey (37°52'15"N, 27°35'58"E) | SWT.GE | Hot spring | 5.8 | 27 | Quartz, feldspars, miche, calcyte |

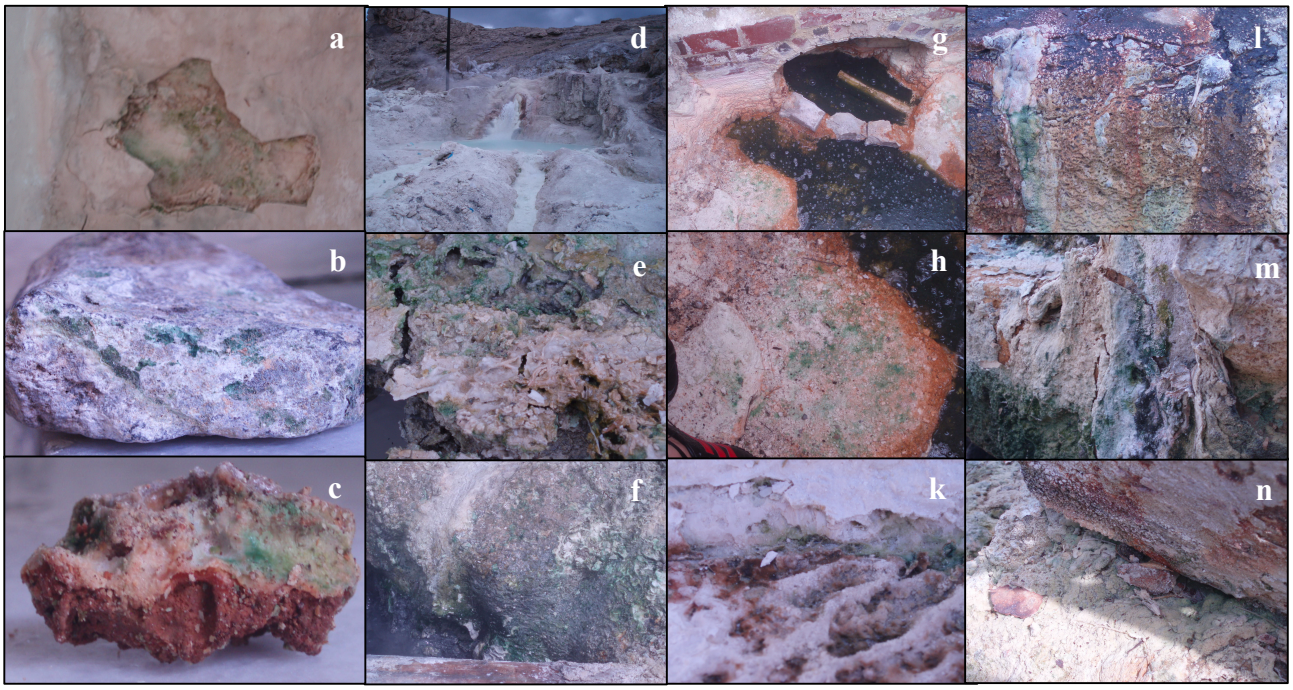
**Table 1.** Location, codes, habitat, pH, temperature and main minerals of sampling sites in Turkey

| Phylotype | | n | v | N | K | h | π | Tajima | Fu and Li F* |
|---|---|---|---|---|---|---|---|---|---|
| ***G.sulphuraria*** | ALL | 136 | 80 | 33 | 19,47 | 0,83±0,028 | 0,0426±0,00356 | -0,35987 | -0,42141 |
| | Italy | 15 | 8 | 7 | 1,29 | 0,724±0,121 | 0,00283±0,0019 | -1,744 | -1,992 |
| | USA | 13 | 5 | 4 | 1,2 | 0,6±0,131 | 0,00269±0,00157 | -0,84 | -1 |
| | Turkey | 10 | 39 | 3 | 17,13 | 0,6±0,131 | 0,0375±0,00482 | -1,17 | 1,3426 |
| | Taiwan | 27 | 39 | 6 | 6,83 | 0,732±0,054 | 0,015±0,0035 | -1,405 | 0,637 |
| | Iceland | 59 | 6 | 7 | 0,267 | 0,224±0,072 | 0,00058±0,000115 | -1,95362 * | -3,337 * * |
| | New Zealand | 10 | 8 | 6 | 2,022 | 0,867±0,085 | 0,0044±0,002 | -1,23 | -1,43 |
| ***G. maxima*** | ALL | 245 | 161 | 100 | 17,3721 | 0,956±0,006 | 0,038±0,0012 | -1,38 | -5,416 ** |
| | Turkey | 40 | 43 | 8 | 8,8 | 0,652±0,069 | 0,02±0,0033 | -0,52 | -2,2 |
| | Japan | 23 | 34 | 8 | 10,52 | 0,861±0,039 | 0,023±0,00345 | -0,302 | -0,815 |
| | Iceland | 24 | 4 | 3 | 0,3333 | 0,163±0,0098 | 0,00073±0,00051 | -1,88381 * | -2,796 * |
| | Taiwan | 149 | 108 | 80 | 17,08 | 0,957±0,009 | 0,0373±0,00067 | -0,6142 | -5,3 ** |
| | New Zealand | 7 | 44 | 4 | 13,05 | 0,81±0,13 | 0,028±0,0059 | -1,58 | -1,836 |
| ***C. merolae*** | ALL | 44 | 19 | 19 | 2,0296 | 0,918 ±0,022 | 0,00443±0,00219 | -1,73184 | -3,456 ** |
| | Turkey | 2 | 1 | 2 | 1 | 1±0,5 | 0,0028±0,00109 | - | - |
| | USA | 35 | 17 | 17 | 2,2454 | 0,934±0,021 | 0,0049±0,00053 | -1,515 | -2,89 * |
| | Italy | 5 | 2 | 3 | 0,8 | 0,7±0,2 | 0,00175±0,00066 | -0,97 | -0,95 |
| ***G.phlegrea*** | ALL | 34 | 26 | 7 | 2,3244 | 0,458±0,104 | 0,0051±0,00272 | -2,3221 ** | -3,4274 ** |
| | Italy | 8 | 4 | 2 | 1 | 0,250±0,180 | 0,0022±0,0016 | -1,5347 | -1,7974 |
| | Turkey | 26 | 24 | 6 | 2,72 | 0,517±0,113 | 0,006±0,0028 | -2,20 ** | -3,267 * * |

Table 2. Summary statistics of rbcL haplotypes for the Turkish cyanidiophycean strains; n . sample size, v . variable sites, N . number of haplotypes, h. haplotype diversity, K. Average number of pairwise nucleotide differences, $\pi$ nucleotide diversity. (significance *: $p< 0.05$; **; $p< 0.10$).

| *G. sulphuraria* | ICE | ITA | NZE | TWN | TUR | USA |
|---|---|---|---|---|---|---|
| **ICE** | *** | 0,839 | 0,844 | 0,87 | 0,55 | 0,91 |
| **ITA** | | *** | 0,71 | 0,88 | 0,14 | 0,85 |
| **NZE** | | | *** | 0,91 | 0,511 | 0,831 |
| **TWN** | | | | *** | 0,67 | 0,97 |
| **TUR** | | | | | *** | 0,68 |
| **USA** | | | | | | *** |

| *G. maxima* | ICE | JAP | TWN | TUR |
|---|---|---|---|---|
| **ICE** | *** | 0,4 | 0,64 | 0,67 |
| **JAP** | | *** | 0,21 | 0,06 |
| **TWN** | | | *** | 0,56 |
| **TUR** | | | | *** |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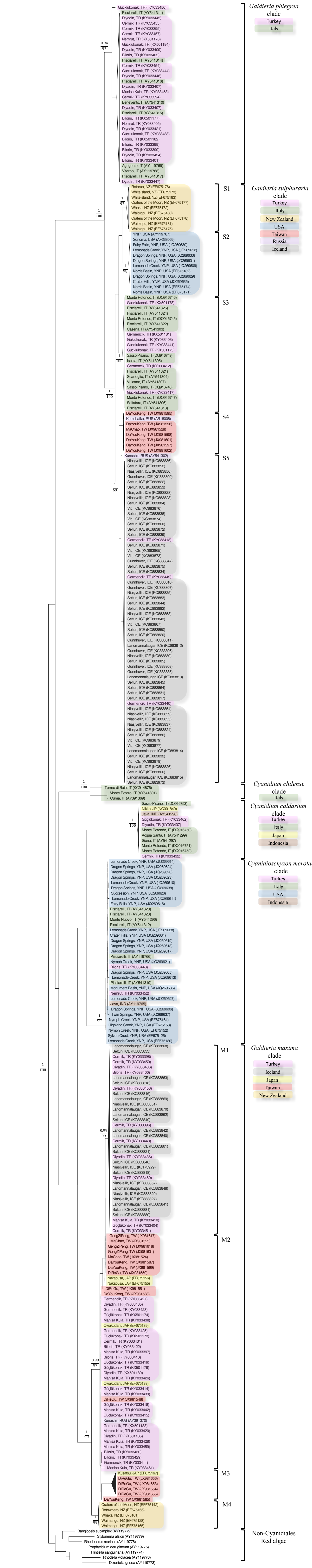
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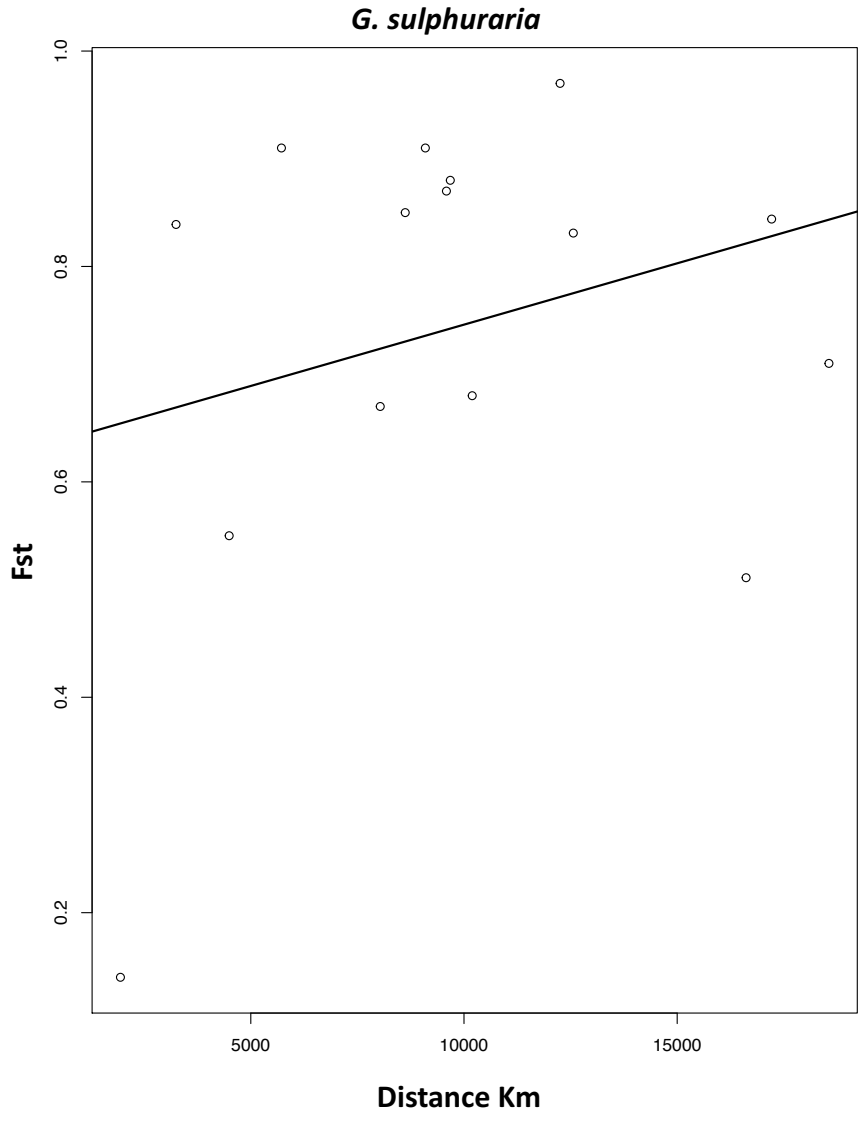
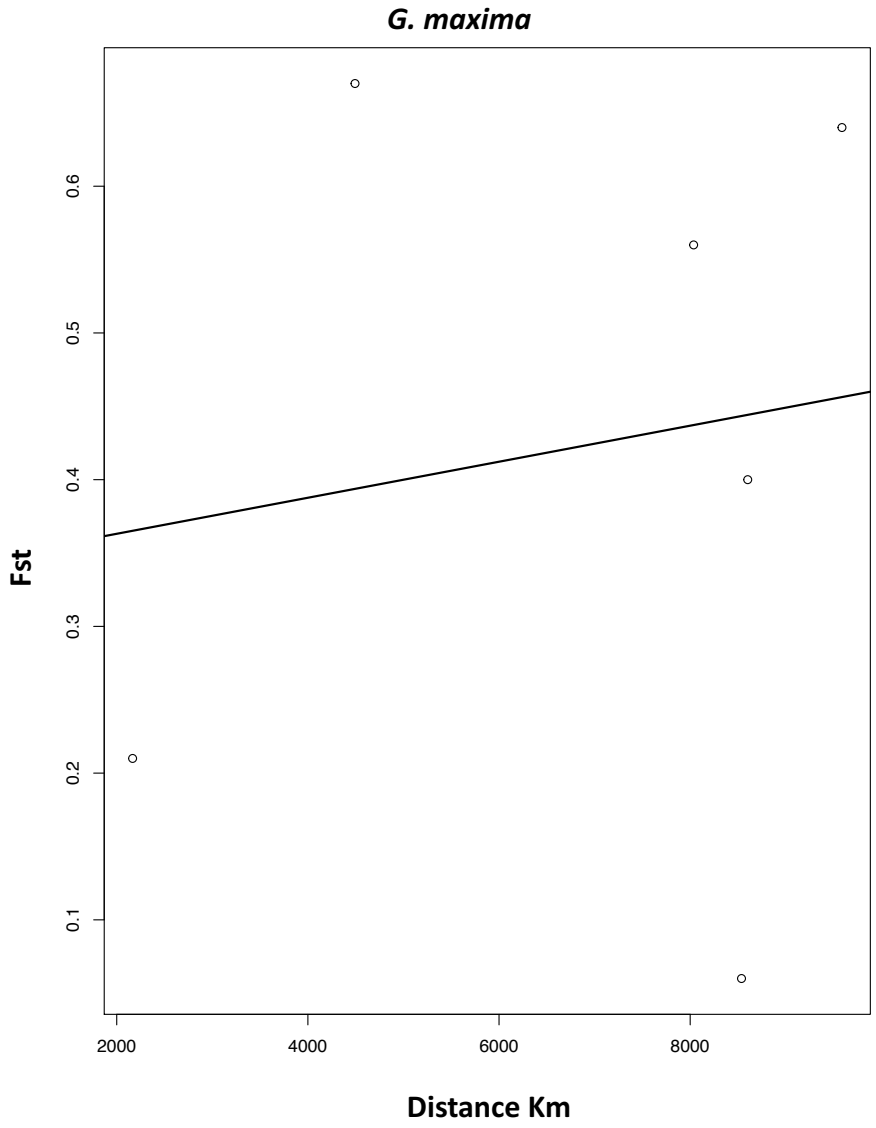
Viti, ICE (KC883878)
Niasjvellir, ICE (KC883826)
Seltun, ICE (KC883866)
Landmannalaugar, ICE (KC883815)
Seltun, ICE (KC883973)
Terme di Baia, IT (KC914876)
Monte Rotaro, IT (AY541301)
Cuma, IT (AY391369)
Sasso Pisano, IT (DQ916753)
Nikko, JP (NC001840)
Java, IND (AY541298)
Güçlükonak, TR (KY033462)
Diyadin, TR (KY033437)
Monte Rotondo, IT (DQ916750)
Acqua Santa, IT (AY541299)
Siena, IT (AY541297)
Monte Rotondo, IT (DQ916751)
Monte Rotondo, IT (DQ916752)
Cermik, TR (KY033432)
Lemonade Creek, YNP, USA (JQ269614)
Dragon Springs, YNP, USA (JQ269624)
Dragon Springs, YNP, USA (JQ269620)
Dragon Springs, YNP, USA (JQ269623)
Lemonade Creek, YNP, USA (JQ269610)
Dragon Springs, YNP, USA (JQ269638)
Succession, YNP, USA (JQ269626)
Lemonade Creek, YNP, USA (JQ269611)
Fairy Falls, YNP, USA (JQ269616)
Pisciarelli, IT (AY541320)
Pisciarelli, IT (AY541323)
Monte Nuovo, IT (AY541296)
Pisciarelli, IT (AY541312)
Lemonade Creek, YNP, USA (JQ269628)
Crater Hills, YNP, USA (JQ269634)
Dragon Springs, YNP, USA (JQ269619)
Dragon Springs, YNP, USA (JQ269618)
Dragon Springs, YNP, USA (JQ269617)
Pisciarelli, IT (AY119766)
Nymph Creek, YNP, USA (JQ269621)
Biloris, TR (KY033448)
Dragon Springs, YNP, USA (JQ269605)
Lemonade Creek, YNP, USA (JQ269613)
Pisciarelli, IT (AY541319)
Monument Basin, YNP, USA (JQ269636)
Nemrut, TR (KY033452)
Lemonade Creek, YNP, USA (JQ269627)
Java, IND (AY119765)
Dragon Springs, YNP, USA (JQ269606)
Twin Springs, YNP, USA (JQ269637)
Nymph Creek, YNP, USA (EF675164)
Highland Creek, YNP, USA (EF675158)
Nymph Creek, YNP, USA (EF675152)
Sylvan Crust, YNP, USA (EF675125)
Lemonade Creek, YNP, USA (EF675130)
Landmannalaugar, ICE (KC883868)
Seltun, ICE (KC883833)
Cermik, TR (KY033398)
Cermik, TR (KY033450)
Diyadin, TR (KY033406)
Biloris, TR (KY033400)
Landmannalaugar, ICE (KC883863)
Seltun, ICE (KC883818)
Diyadin, TR (KY033453)
Seltun, ICE (KC883816)
Landmannalaugar, ICE (KC883869)
Niasjvellir, ICE (KC883851)
Landmannalaugar, ICE (KC883870)
Landmannalaugar, ICE (KC883862)
Seltun, ICE (KC883849)
Cermik, TR (KY033396)
Landmannalaugar, ICE (KC883842)
Landmannalaugar, ICE (KC883840)
Cermik, TR (KY033443)
Landmannalaugar, ICE (KC883861)
Seltun, ICE (KC883821)
Diyadin, TR (KY033436)
Seltun, ICE (KC883846)
Niasjvellir, ICE (KJ173929)
Seltun, ICE (KC883818)
Diyadin, TR (KY033460)
Niasjvellir, ICE (KC883857)
Landmannalaugar, ICE (KC883848)
Niasjvellir, ICE (KC883829)
Niasjvellir, ICE (KC883827)
Landmannalaugar, ICE (KC883841)
Seltun, ICE (KC883881)
Seltun, ICE (KC883880)
Manisa Kula, TR (KY033410)
Güçlükonak, TR (KY033404)
Cermik, TR (KY033451)
GengZiPeng, TW (JX981617)
MaChao, TW (JX981525)
GengZiPeng, TW (JX981618)
GengZiPeng, TW (JX981631)
MaChao, TW (JX981524)
DaYouKeng, TW (JX981587)
DaYouKeng, TW (JX981599)
DiReGu, TW (JX981550)
Nakabusa, JAP (EF675156)
Nakabusa, JAP (EF675155)
DiReGu, TW (JX981551)
DaYouKeng, TW (JX981583)
Germencik, TR (KY033427)
Diyadin, TR (KY033435)
Germencik, TR (KY033423)
Güçlükonak, TR (KX501174)
Manisa Kula, TR (KY033438)
Owakudani, JAP (EF675139)
Germencik, TR (KY033425)
Güçlükonak, TR (KX501173)
Cermik, TR (KY033431)
Biloris, TR (KY033422)
Manisa Kula, TR (KY033397)
Biloris, TR (KY033416)
Güçlükonak, TR (KY033419)
Güçlükonak, TR (KX501179)
Diyadin, TR (KX501180)
Manisa Kula, TR (KY033426)
Owakudani, JAP (EF675138)
Güçlükonak, TR (KY033414)
Manisa Kula, TR (KY033439)
DiReGu, TW (JX981548)
Güçlükonak, TR (KY033418)
Manisa Kula, TR (KY033442)
Güçlükonak, TR (KY033415)
Kunashir, RUS (AY391370)
Germencik, TR (KX501183)
Manisa Kula, TR (KY033420)
Diyadin, TR (KX501185)
Manisa Kula, TR (KY033428)
Manisa Kula, TR (KY033459)
Biloris, TR (KY033430)
Biloris, TR (KY033429)
Germencik, TR (KY033411)
Manisa Kula, TR (KY033461)
Kusatsu, JAP (EF675167)
DiReGu, TW (JX981656)
DiReGu, TW (JX981653)
DiReGu, TW (JX981654)
DiReGu, TW (JX981655)
DaYouKeng, TW (JX981585)
Craters of the Moon, NZ (EF675142)
Rotowhero, NZ (EF675166)
Whaka, NZ (EF675161)
Waimangu, NZ (EF675128)
Waimangu, NZ (EF675165)
Bangiopsis subimplex (AY119772)
Stylonema alsidii (AY119779)
Rhodosorus marinus (AY119778)
Porphyridium aerugineum (AY119775)
Flintiella sanguinaria (AY119774)
Rhodella violacea (AY119776)
Dixoniella grisea (AY119773)
0.94/97
1/100
1/97
1/98
1/100
1/100
1/69
1/100
1/100
0.99/99
1/99
0.99/97
1/99
1/99
S1
S2
S3
S4
S5
M1
M2
M3
M4
Galdieria phlegrea clade
Turkey
Italy
Galdieria sulphuraria clade
Turkey
Italy
New Zealand
USA
Taiwan
Russia
Iceland
Cyanidium chilense clade
Italy
Cyanidium caldarium clade
Turkey
Italy
Japan
Indonesia
Cyanidioschyzon merolae clade
Turkey
Italy
USA
Indonesia
Galdieria maxima clade
Turkey
Iceland
Japan
Taiwan
New Zealand
Non-Cyanidiales Red algae


G. sulphuraria
Fst
Distance Km
1.0
0.8
0.6
0.4
0.2
5000
10000
15000


G. maxima
Fst
Distance Km
0.6
0.5
0.4
0.3
0.2
0.1
2000
4000
6000
8000

| Taxa | Strain | Sampling Site | GenBank number |
|---|---|---|---|
| *Cyanidiales sp.* | CHJ-4 | USA, Crater Hills, YNP | JQ269635 |
| | CHJ-5 | USA, Crater Hills, YNP | JQ269634 |
| | DS1-6 | USA, Dragon Springs, YNP | JQ269623 |
| | DS1-9 | USA, Dragon Springs, YNP | JQ269631 |
| | DS2-2 | USA, Dragon Springs, YNP | JQ269638 |
| | DS2-5 | USA, Dragon Springs, YNP | JQ269629 |
| | DS3-1 | USA, Dragon Springs, YNP | JQ269633 |
| | DS3-3 | USA, Dragon Springs, YNP | JQ269624 |
| | DSB-9 | USA, Dragon Springs, YNP | JQ269617 |
| | DSC-8 | USA, Dragon Springs, YNP | JQ269618 |
| | DSD-7 | USA, Dragon Springs, YNP | JQ269605 |
| | DSE-8 | USA, Dragon Springs, YNP | JQ269619 |
| | DSF-12 | USA, Dragon Springs, YNP | JQ269620 |
| | DSH-4 | USA, Dragon Springs, YNP | JQ269606 |
| | SFFL-8 | USA, Fairy Falls, YNP | JQ269630 |
| | SFFR-7 | USA, Fairy Falls, YNP | JQ269616 |
| | SFFL-5 | USA, Fairy Falls, YNP | JQ269630 |
| | LCBCEL-7 | USA, Lemonade Creek, YNP | JQ269610 |
| | LCBTERR-12 | USA, Lemonade Creek, YNP | JQ269611 |
| | LCCYEGR-4 | USA, Lemonade Creek, YNP | JQ269614 |
| | LCASUB-11 | USA, Lemonade Creek, YNP | JQ269627 |
| | LCBSUB-5 | USA, Lemonade Creek, YNP | JQ269628 |
| | LCCBLGR-8 | USA, Lemonade Creek, YNP | JQ269613 |
| | LCBTERR-6 | USA, Lemonade Creek, YNP | JQ269612 |
| | LCBCEL-5 | USA, Lemonade Creek, YNP | JQ269609 |
| | RIVER1B-5 | USA, Monument Basin, YNP | JQ269636 |
| | NCB-4 | USA, Nymph Creek, YNP | JQ269621 |
| | SSI-6 | USA,Succession, YNP | JQ269622 |
| . | SSII-1 | USA,Succession, YNP | JQ269626 |
| | TS-4 | USA, Twin, YNP | JQ269637 |
| *Cyanidioschyzon merolae* | ACUF201 | Indonesia, Java | AY119765 |
| | ACUF202 | Italy, Monte Nuovo | AY541296 |
| | ACUF001 | Italy, Pisciarelli | AY119766 |
| | Clone-C16 | Italy, Pisciarelli | AY541319 |
| | CloneA1 | Italy, Pisciarelli | AY541312 |
| | CloneD1 | Italy, Pisciarelli | AY541320 |
| | CloneE10 | Italy, Pisciarelli | AY541323 |
| | 10D | Italy, Sardinia | D63675 |
| | 10D | Italy, Sardinia | NC_004799 |
| | CCMEE5625 | USA, Highland Creek, YNP | EF675158 |
| | CCMEE5576 | USA, Lemonade Creek, YNP | EF675130 |
| | CCMEE5506 | USA, Norris Basin, YNP | EF675146 |
| | CCMEE5507 | USA, Norris Basin, YNP | EF675160 |
| | CCMEE5631 | USA, Norris Basin, YNP | EF675140 |
| | CCMEE5639 | USA, Norris Basin, YNP | EF675127 |
| | CCMEE5640 | USA, Norris Basin, YNP | EF675137 |
| | CCMEE5584 | USA, Nymph Creek, YNP | EF675164 |
| | CCMEE5585 | USA, Nymph Creek, YNP | EF675152 |
| | CCMEE5593 | USA, Obsidian Creek, YNP | EF675124 |
| | CCMEE5610 | USA, Sylvan Crust, YNP | EF675125 |
| | CCMEE5609 | USA, Sylvan Springs, YNP | EF675144 |
| *Cyanidium caldarium* | ACUF182 | Indonesia, Java | AY541298 |
| | ACUF 020 | Italy, Acqua Santa | AY541299 |
| | isolate MR4-22 | Italy, Monte Rotondo | DQ916750 |
| | isolate MR5-5 | Italy, Monte Rotondo | DQ916751 |
| | isolate MR6-C35 | Italy, Monte Rotondo | DQ916752 |
| | Clone C2 | Italy, Pisciarelli | AY541318 |
| | isolate SP1-10 | Italy, Sasso Pisano | DQ916753 |
| | ACUF019 | Italy, Siena | AY541297 |
| | RK1 | Japan, Nikko | NC_001840 |
| *Galdieria daedala* | IPPAS P508 | Russia, Kunashir | AY541302 |

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|---|---|---|---|
| | ACUF419 | Iceland, Landmannalaugar | KC883840 |
| | ACUF420 | Iceland, Landmannalaugar | KC883841 |
| | ACUF421 | Iceland, Landmannalaugar | KC883842 |
| | ACUF428 | Iceland, Landmannalaugar | KC883848 |
| | ACUF449 | Iceland, Landmannalaugar | KC883861 |
| | ACUF450 | Iceland, Landmannalaugar | KC883862 |
| | ACUF451 | Iceland, Landmannalaugar | KC883863 |
| | ACUF456 | Iceland, Landmannalaugar | KC883868 |
| | ACUF457 | Iceland, Landmannalaugar | KC883869 |
| | ACUF458 | Iceland, Landmannalaugar | KC883870 |
| | ACUF404 | Iceland, Niasjvellir | KC883827 |
| | ACUF406 | Iceland, Niasjvellir | KJ173929 |
| | ACUF407 | Iceland, Niasjvellir | KC883829 |
| | ACUF438 | Iceland, Niasjvellir | KC883851 |
| | ACUF445 | Iceland, Niasjvellir | KC883857 |
| | ACUF389 | Iceland, Seltun | KC883816 |
| | ACUF392 | Iceland, Seltun | KC883818 |
| | ACUF393 | Iceland, Seltun | KC883819 |
| | ACUF396 | Iceland, Seltun | KC883821 |
| | ACUF411 | Iceland, Seltun | KC883833 |
| | ACUF425 | Iceland, Seltun | KC883846 |
| | ACUF436 | Iceland, Seltun | KC883849 |
| | ACUF468 | Iceland, Seltun | KC883880 |
| | ACUF469 | Iceland, Seltun | KC883881 |
| | CCMEE5664 | Japan, Kusatsu | EF675145 |
| ***Galdieria maxima*** | CCMEE5665 | Japan, Kusatsu | EF675129 |
| | CCMEE5667 | Japan, Kusatsu | EF675151 |
| | CCMEE5676 | Japan, Kusatsu | EF675143 |
| | CCMEE5677 | Japan, Kusatsu | EF675132 |
| | CCMEE5678 | Japan, Kusatsu | EF675168 |
| | CCMEE5679 | Japan, Kusatsu | EF675163 |
| | CCMEE5680 | Japan, Kusatsu | EF675167 |
| | CCMEE5681 | Japan, Kusatsu | EF675157 |
| | CCMEE5660 | Japan, Nakabusa | EF675156 |
| | CCMEE5661 | Japan, Nakabusa | EF675150 |
| | CCMEE5662 | Japan, Nakabusa | EF675154 |
| | CCMEE5663 | Japan, Nakabusa | EF675159 |
| | CCMEE5674 | Japan, Nakabusa | EF675153 |
| | CCMEE5675 | Japan, Nakabusa | EF675155 |
| | CCMEE5657 | Japan, Owakudani | EF675139 |
| | CCMEE5658 | Japan, Owakudani | EF675162 |
| | CCMEE5659 | Japan, Owakudani | EF675138 |
| | CCMEE5669 | Japan, Owakudani | EF675126 |
| | CCMEE5670 | Japan, Owakudani | EF675148 |
| | CCMEE5672 | Japan, Owakudani | EF675131 |
| | CCMEE5673 | Japan, Owakudani | EF675141 |
| | CCMEE5705 | New Zealand, Rotowhero | EF675166 |
| | CCMEE5703 | New Zealand, Waimangu | EF675165 |
| | CCMEE5704 | New Zealand, Waimangu | EF675128 |
| | CCMEE5713 | New Zealand, Waiotopu | EF675147 |
| | CCMEE5709 | New Zealand, Whaka | EF675161 |
| | CCMEE5715 | New Zealand, Whaka | EF675149 |
| | CCMEE5720 | New Zealand, White Island | EF675134 |
| | CCMEE5716 | New Zealand, Craters of the | EF675142 |
| | IPPAS P507 | Russia, Kunashir | AY391370 |
| ***Galdieria partita*** | IPPAS P500 | Russia, Kamchatka | AB18008 |
| | ACUF063 | Italy, Agrigento | AY119769 |
| | ACUF012 | Italy, Benevento | AY541310 |
| | ACUF002 | Italy, Pisciarelli | AY541311 |
| ***Galdieria phlegrea*** | CloneB15 | Italy, Pisciarelli | AY541314 |
| | CloneB19 | Italy, Pisciarelli | AY541315 |
| | CloneB20 | Italy, Pisciarelli | AY541316 |

| | | | |
|---|---|---|---|
| | CloneC1 | Italy, Pisciarelli | AY541317 |
| ***Galdieria phlegrea*** | ACUF009 | Italy, Viterbo | AY119768 |
| ***Galdieria sulphuraria*** | ACUF376 | Iceland, Gunnhuver | KC883806 |
| | ACUF380 | Iceland, Gunnhuver | KC883807 |
| | ACUF381 | Iceland, Gunnhuver | KC883808 |
| | ACUF382 | Iceland, Gunnhuver | KC883809 |
| | ACUF383 | Iceland, Gunnhuver | KC883810 |
| | ACUF384 | Iceland, Gunnhuver | KC883811 |
| | ACUF413 | Iceland, Gunnhuver | KC883835 |
| | ACUF427 | Iceland, Gunnhuver | KC883847 |
| | ACUF385 | Iceland, Landmannalaugar | KC883812 |
| | ACUF386 | Iceland, Landmannalaugar | KC883813 |
| | ACUF387 | Iceland, Landmannalaugar | KC883814 |
| | ACUF388 | Iceland, Landmannalaugar | KC883815 |
| | ACUF399 | Iceland, Niasjvellir | KC883823 |
| | ACUF400 | Iceland, Niasjvellir | KC883824 |
| | ACUF402 | Iceland, Niasjvellir | KC883825 |
| | ACUF403 | Iceland, Niasjvellir | KC883826 |
| | ACUF405 | Iceland, Niasjvellir | KC883828 |
| | ACUF408 | Iceland, Niasjvellir | KC883830 |
| | ACUF414 | Iceland, Niasjvellir | KC883836 |
| | ACUF415 | Iceland, Niasjvellir | KC883837 |
| | ACUF442 | Iceland, Niasjvellir | KC883854 |
| | ACUF443 | Iceland, Niasjvellir | KC883855 |
| | ACUF444 | Iceland, Niasjvellir | KC883856 |
| | ACUF446 | Iceland, Niasjvellir | KC883858 |
| | ACUF447 | Iceland, Niasjvellir | KC883859 |
| | ACUF390 | Iceland, Seltun | KC883817 |
| | ACUF395 | Iceland, Seltun | KC883820 |
| | ACUF397 | Iceland, Seltun | KC883822 |
| | ACUF398 | Iceland, Seltun | KC883973 |
| | ACUF409 | Iceland, Seltun | KC883831 |
| | ACUF410 | Iceland, Seltun | KC883832 |
| | ACUF412 | Iceland, Seltun | KC883834 |
| | ACUF416 | Iceland, Seltun | KC883838 |
| | ACUF417 | Iceland, Seltun | KC883839 |
| | ACUF422 | Iceland, Seltun | KC883843 |
| | ACUF423 | Iceland, Seltun | KC883844 |
| | ACUF424 | Iceland, Seltun | KC883845 |
| | ACUF437 | Iceland, Seltun | KC883850 |
| | ACUF439 | Iceland, Seltun | KC883852 |
| | ACUF440 | Iceland, Seltun | KC883853 |
| | ACUF448 | Iceland, Seltun | KC883860 |
| | ACUF452 | Iceland, Seltun | KC883864 |
| | ACUF454 | Iceland, Seltun | KC883866 |
| | ACUF459 | Iceland, Seltun | KC883871 |
| | ACUF460 | Iceland, Seltun | KC883872 |
| | ACUF463 | Iceland, Seltun | KC883875 |
| | ACUF470 | Iceland, Seltun | KC883882 |
| | ACUF472 | Iceland, Seltun | KC883883 |
| | ACUF473 | Iceland, Seltun | KC883884 |
| | ACUF474 | Iceland, Seltun | KC883885 |
| | ACUF475 | Iceland, Seltun | KC883886 |
| | ACUF453 | Iceland, Viti | KC883865 |
| | ACUF455 | Iceland, Viti | KC883867 |
| | ACUF461 | Iceland, Viti | KC883873 |
| | ACUF462 | Iceland, Viti | KC883874 |
| | ACUF464 | Iceland, Viti | KC883876 |
| | ACUF465 | Iceland, Viti | KC883877 |
| | ACUF466 | Iceland, Viti | KC883878 |
| | ACUF467 | Iceland, Viti | KC883879 |
| | ACUF011 | Italy, Caserta | AY541303 |
| | ACUF015 | Italy, Ischia | AY541305 |
| | isolate MR4-21 | Italy, Monte Rotondo | DQ916745 |
| | isolate MR5- C17 | Italy, Monte Rotondo | DQ916746 |
| | isolate MR6- C36 | Italy, Monte Rotondo | DQ916747 |
| | CloneA12 | Italy, Pisciarelli | AY541313 |
| | CloneD15 | Italy, Pisciarelli | AY541322 |
| | CloneD5 | Italy, Pisciarelli | AY541321 |
| | CloneE11 | Italy, Pisciarelli | AY541324 |
| | CloneE12 | Italy, Pisciarelli | AY541325 |
| | isolate SP1-10 | Italy, Sasso Pisano | DQ916748 |
| | isolate SP3-C2 | Italy, Sasso Pisano | DQ916749 |

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|---|---|---|---|
| | ACUF018 | Italy, Scarfoglio | AY541304 |
| ***Galdieria sulphuraria*** | ACUF017 | Italy, Solfatara | AY541306 |
| | ACUF021 | Italy, Vulcano | AY541307 |
| | CCMEE5706 | New Zealand, Craters of the Moon | EF675177 |
| | CCMEE5712 | New Zealand, Craters of the Moon | EF675178 |
| | CCMEE5717 | New Zealand, Rotorua | EF675176 |
| | CCMEE5707 | New Zealand, Waiotopu | EF675181 |
| | CCMEE5714 | New Zealand, Waiotopu | EF675180 |
| | CCMEE5719 | New Zealand, Waiotopu | EF675175 |
| | CCMEE5718 | New Zealand, Whaka | EF675179 |
| | CCMEE5708 | New Zealand, Whaka | EF675172 |
| | CCMEE5710 | New Zealand, WhiteIsland | EF675183 |
| | CCMEE5711 | New Zealand, WhiteIsland | EF675173 |
| | LCATERR-7 | USA, Lemonade Creek, YNP | JQ269608 |
| | CCMEE5511 | USA, Norris Basin, YNP | EF675174 |
| | CCMEE5572 | USA, Norris Basin, YNP | EF675182 |
| | CCMEE5573 | USA, Norris Basin, YNP | EF675171 |
| | UTEX2393 | USA, Sonoma, California | AF233069 |
| | SAG 108.79 | USA, Yellowstone | AY119767 |
| ***Cyanidium chilense*** | Sybil cave | Italy, Cuma | AY391369 |
| | sp.19 | Italy, Monte Rotaro | AY541300 |
| | sp.20 | Italy, Monte Rotaro | AY541301 |
| | | Italy, Terme di baia | KC914876 |
| ***Galdieria sp.*** | clone 12.ENVS.DYK.ditch60.1.1.1 | Taiwan, DaYouKeng | JX981552 |
| | clone 12.ENVS.DYK.ditch60.1.1.2 | Taiwan, DaYouKeng | JX981553 |
| | clone 12.ENVS.DYK.ditch60.1.1.3 | Taiwan, DaYouKeng | JX981554 |
| | clone 12.ENVS.DYK.ditch60.1.1.5 | Taiwan, DaYouKeng | JX981555 |
| | clone 12.ENVS.DYK.ditch60.1.1.6 | Taiwan, DaYouKeng | JX981556 |
| | clone 12.ENVS.DYK.ditch60.1.1.7 | Taiwan, DaYouKeng | JX981557 |
| | clone 12.ENVS.DYK.ditch60.1.1.9 | Taiwan, DaYouKeng | JX981559 |
| | clone 12.ENVS.DYK.ditch60.1.1.11 | Taiwan, DaYouKeng | JX981561 |
| | clone 12.ENVS.DYK.ditch60.1.1.12 | Taiwan, DaYouKeng | JX981562 |
| | clone 12.ENVS.DYK.ditch60.1.1.15 | Taiwan, DaYouKeng | JX981563 |
| | clone 12.ENVS.DYK.ditch60.1.2.3 | Taiwan, DaYouKeng | JX981564 |
| | clone 12.ENVS.DYK.ditch60.1.2.6 | Taiwan, DaYouKeng | JX981565 |
| | clone 12.ENVS.DYK.ditch60.1.2.8 | Taiwan, DaYouKeng | JX981566 |
| | clone 12.ENVS.DYK.ditch60.1.3.5 | Taiwan, DaYouKeng | JX981568 |
| | clone12.ENVS.DYK.ditch45.2.2 | Taiwan, DaYouKeng | JX981569 |
| | clone12.ENVS.DYK.ditch45.2.3 | Taiwan, DaYouKeng | JX981570 |
| | clone12.ENVS.DYK.ditch45.2.5 | Taiwan, DaYouKeng | JX981571 |
| | clone12.ENVS.DYK.ditch45.2.6 | Taiwan, DaYouKeng | JX981572 |
| | clone12.ENVS.DYK.ditch45.2.7 | Taiwan, DaYouKeng | JX981573 |
| | clone12.ENVS.DYK.ditch45.2.8 | Taiwan, DaYouKeng | JX981574 |
| | clone12.ENVS.DYK.ditch45.2.9 | Taiwan, DaYouKeng | JX981575 |
| | clone12.ENVS.DYK.ditch45.2.10 | Taiwan, DaYouKeng | JX981576 |
| | clone12.ENVS.DYK.ditch45.2.12 | Taiwan, DaYouKeng | JX981577 |
| | clone12.ENVS.DYK.ditch45.2.13 | Taiwan, DaYouKeng | JX981578 |
| | clone12.ENVS.DYK.ditch45.2.14 | Taiwan, DaYouKeng | JX981579 |
| | clone12.ENVS.DYK.ditch45.2.15 | Taiwan, DaYouKeng | JX981580 |
| | clone12.ENVS.DYK.ditch45.4.1 | Taiwan, DaYouKeng | JX981581 |
| | clone12.ENVS.DYK.ditch45.4.6 | Taiwan, DaYouKeng | JX981583 |
| | clone12.ENVS.DYK.ditch45.4.8 | Taiwan, DaYouKeng | JX981585 |
| | clone12.ENVS.DYK.endolithic.2 | Taiwan, DaYouKeng | JX981586 |
| | clone12.ENVS.DYK.endolithic.4 | Taiwan, DaYouKeng | JX981587 |
| | clone12.ENVS.DYK.endolithic.6 | Taiwan, DaYouKeng | JX981588 |
| | clone12.ENVS.DYK.endolithic.7 | Taiwan, DaYouKeng | JX981589 |
| | clone12.ENVS.DYK.endolithic.8 | Taiwan, DaYouKeng | JX981590 |
| | clone12.ENVS.DYK.endolithic.10 | Taiwan, DaYouKeng | JX981591 |
| | clone12.ENVS.DYK.endolithic.11 | Taiwan, DaYouKeng | JX981592 |
| | clone12.ENVS.DYK.endolithic.12 | Taiwan, DaYouKeng | JX981593 |
| | clone12.ENVS.DYK.endolithic.13 | Taiwan, DaYouKeng | JX981594 |
| | clone12.ENVS.DYK.endolithic.14 | Taiwan, DaYouKeng | JX981595 |
| | clone12.ENVS.DYK.endolithic.15 | Taiwan, DaYouKeng | JX981596 |
| | clone12.ENVS.DYK.endolithic.16 | Taiwan, DaYouKeng | JX981597 |
| | clone12.ENVS.DYK.endolithic.17 | Taiwan, DaYouKeng | JX981598 |
| | clone12.ENVS.DYK.endolithic.18 | Taiwan, DaYouKeng | JX981599 |
| | clone12.ENVS.DYK.endolithic.21 | Taiwan, DaYouKeng | JX981600 |
| | clone12.ENVS.DYK.endolithic.22 | Taiwan, DaYouKeng | JX981601 |
| | clone12.ENVS.DYK.endolithic.23 | Taiwan, DaYouKeng | JX981602 |
| | clone12.ENVS.DYK.endolithic.24 | Taiwan, DaYouKeng | JX981603 |
| | clone12.ENVS.DYK.endolithic.25 | Taiwan, DaYouKeng | JX981604 |
| | clone12.ENVS.DYK.endolithic.26 | Taiwan, DaYouKeng | JX981605 |
| | clone12.ENVS.DYK.endolithic.28 | Taiwan, DaYouKeng | JX981606 |

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| ***Galdieria sp.*** | clone12.ENVS.DYK.endolithic.29 | Taiwan, DaYouKeng | JX981607 |
| | clone12.ENVS.DYK.endolithic.30 | Taiwan, DaYouKeng | JX981608 |
| | THAL006.DYK01.Gp | Taiwan, DaYouKeng | KJ125469 |
| | THAL007.DYK02.Gp | Taiwan, DaYouKeng | KJ125470 |
| | clone12.ENVS.DRG.stream40.sun.1.3 | Taiwan, DiReGu | JX981533 |
| | clone12.ENVS.DRG.stream40.sun.2.2 | Taiwan, DiReGu | JX981534 |
| | clone12.ENVS.DRG.stream40.sun.2.5 | Taiwan, DiReGu | JX981536 |
| | clone12.ENVS.DRG.stream40.sun.3.1 | Taiwan, DiReGu | JX981537 |
| | clone12.ENVS.DRG.stream40.sun.3.2 | Taiwan, DiReGu | JX981538 |
| | clone12.ENVS.DRG.stream40.sun.3.3 | Taiwan, DiReGu | JX981539 |
| | clone12.ENVS.DRG.stream40.sun.3.7 | Taiwan, DiReGu | JX981540 |
| | clone12.ENVS.DRG.stream40.sun.3.13 | Taiwan, DiReGu | JX981541 |
| | clone12.ENVS.DRG.stream40.sun.3.14 | Taiwan, DiReGu | JX981542 |
| | clone12.ENVS.DRG.stream40.sun.3.15 | Taiwan, DiReGu | JX981543 |
| | clone12.ENVS.DRG.stream40.sun.3.20 | Taiwan, DiReGu | JX981546 |
| | clone12.ENVS.DRG.stream40.sun.4.6 | Taiwan, DiReGu | JX981548 |
| | clone12.ENVS.DRG.stream40.sun.4.9 | Taiwan, DiReGu | JX981549 |
| | clone12.ENVS.DRG.stream40.sun.4.10 | Taiwan, DiReGu | JX981550 |
| | clone12.ENVS.DRG.stream40.sun.4.15 | Taiwan, DiReGu | JX981551 |
| | clone 05.ENVS.DRG.stream42.sun.2 | Taiwan, DiReGu | JX981643 |
| | clone 05.ENVS.DRG.stream42.sun.3 | Taiwan, DiReGu | JX981644 |
| | clone 05.ENVS.DRG.stream42.sun.4 | Taiwan, DiReGu | JX981645 |
| | clone 05.ENVS.DRG.stream42.sun.5 | Taiwan, DiReGu | JX981646 |
| | clone 05.ENVS.DRG.stream42.sun.6 | Taiwan, DiReGu | JX981647 |
| | clone 05.ENVS.DRG.stream42.sun.7 | Taiwan, DiReGu | JX981648 |
| | clone 05.ENVS.DRG.stream42.sun.8 | Taiwan, DiReGu | JX981649 |
| | clone 05.ENVS.DRG.stream42.sun.9 | Taiwan, DiReGu | JX981650 |
| | clone 05.ENVS.DRG.stream42.sun.10 | Taiwan, DiReGu | JX981651 |
| | clone 05.ENVS.DRG.stream42.sun.11 | Taiwan, DiReGu | JX981652 |
| | clone 05.ENVS.DRG.stream42.sun.12 | Taiwan, DiReGu | JX981653 |
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| | clone 05.ENVS.DRG.stream42.sun.14 | Taiwan, DiReGu | JX981655 |
| | clone 05.ENVS.DRG.stream42.sun.15 | Taiwan, DiReGu | JX981656 |
| | clone 05.ENVS.DRG.stream42.sun.16 | Taiwan, DiReGu | JX981657 |
| | clone 05.ENVS.DRG.stream42.shaded.1 | Taiwan, DiReGu | JX981658 |
| | clone 05.ENVS.DRG.stream42.shaded.2 | Taiwan, DiReGu | JX981659 |
| | clone 05.ENVS.DRG.stream42.shaded.3 | Taiwan, DiReGu | JX981660 |
| | clone 05.ENVS.DRG.stream42.shaded.4 | Taiwan, DiReGu | JX981661 |
| | clone 05.ENVS.DRG.stream42.shaded.6 | Taiwan, DiReGu | JX981663 |
| | clone 05.ENVS.DRG.stream42.shaded.7 | Taiwan, DiReGu | JX981664 |
| | clone 05.ENVS.DRG.stream42.shaded.8 | Taiwan, DiReGu | JX981665 |
| | clone 05.ENVS.DRG.stream42.shaded.9 | Taiwan, DiReGu | JX981666 |
| | clone 05.ENVS.DRG.stream42.shaded.10 | Taiwan, DiReGu | JX981667 |
| | clone 05.ENVS.DRG.stream42.shaded.11 | Taiwan, DiReGu | JX981668 |
| | clone 05.ENVS.DRG.stream42.shaded.12 | Taiwan, DiReGu | JX981669 |
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| | clone 05.ENVS.DRG.stream42.shaded.14 | Taiwan, DiReGu | JX981671 |
| | clone 05.ENVS.DRG.stream42.shaded.15 | Taiwan, DiReGu | JX981672 |
| | clone 05.ENVS.DRG.stream42.shaded.16 | Taiwan, DiReGu | JX981673 |
| | clone 05.ENVS.DRG.stream42.shaded.17 | Taiwan, DiReGu | JX981674 |
| | clone 05.ENVS.DRG.stream42.shaded.18 | Taiwan, DiReGu | JX981675 |
| | THAL001.DRG01.Gp | Taiwan, DiReGu | KJ125464 |
| | THAL002.DRG02.Gp | Taiwan, DiReGu | KJ125465 |
| | THAL003.DRG03.Gp | Taiwan, DiReGu | KJ125466 |
| | THAL004.DRG04.Gp | Taiwan, DiReGu | KJ125467 |
| | THAL008.DRG05.Gp | Taiwan, DiReGu | KJ125471 |
| | THAL005.GZP01.Gp | Taiwan, GengZiPeng | KJ125468 |
| | clone 12.ENVS.GZP.epilithic.1 | Taiwan, GengZiPeng | JX981624 |
| | clone 12.ENVS.GZP.epilithic.2 | Taiwan, GengZiPeng | JX981625 |
| | clone 12.ENVS.GZP.epilithic.3 | Taiwan, GengZiPeng | JX981626 |
| | clone 12.ENVS.GZP.epilithic.4 | Taiwan, GengZiPeng | JX981627 |
| | clone 12.ENVS.GZP.epilithic.5 | Taiwan, GengZiPeng | JX981628 |
| | clone 12.ENVS.GZP.epilithic.6 | Taiwan, GengZiPeng | JX981629 |
| | clone 12.ENVS.GZP.epilithic.7 | Taiwan, GengZiPeng | JX981630 |
| | clone 12.ENVS.GZP.epilithic.8 | Taiwan, GengZiPeng | JX981631 |
| | clone 12.ENVS.GZP.epilithic.9 | Taiwan, GengZiPeng | JX981632 |
| | clone 12.ENVS.GZP.epilithic.10 | Taiwan, GengZiPeng | JX981633 |
| | clone 12.ENVS.GZP.epilithic.12 | Taiwan, GengZiPeng | JX981634 |
| | clone 12.ENVS.GZP.epilithic.13 | Taiwan, GengZiPeng | JX981635 |
| | clone 12.ENVS.GZP.epilithic.14 | Taiwan, GengZiPeng | JX981636 |
| | clone 12.ENVS.GZP.epilithic.16 | Taiwan, GengZiPeng | JX981638 |
| | clone 12.ENVS.GZP.epilithic.17 | Taiwan, GengZiPeng | JX981639 |
| | clone 12.ENVS.GZP.epilithic.18 | Taiwan, GengZiPeng | JX981640 |

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|---|---|---|---|
| | clone 12.ENVS.GZP.epilithic.19 | Taiwan, GengZiPeng | JX981641 |
| | clone12.ENVS.GZP.epilithic.low1 | Taiwan, GengZiPeng | KC313262 |
| | clone12.ENVS.GZP.epilithic.low2 | Taiwan, GengZiPeng | KC313263 |
| | clone12.ENVS.GZP.epilithic.low3 | Taiwan, GengZiPeng | KC313264 |
| | clone12.ENVS.GZP.epilithic.low4 | Taiwan, GengZiPeng | KC313265 |
| | clone12.ENVS.GZP.epilithic.low5 | Taiwan, GengZiPeng | KC313266 |
| | clone12.ENVS.GZP.epilithic.low6 | Taiwan, GengZiPeng | KC313267 |
| | clone12.ENVS.GZP.epilithic.low7 | Taiwan, GengZiPeng | KC313268 |
| | clone12.ENVS.GZP.epilithic.low8 | Taiwan, GengZiPeng | KC313269 |
| | clone12.ENVS.GZP.epilithic.low9 | Taiwan, GengZiPeng | KC313270 |
| | clone12.ENVS.GZP.epilithic.low10 | Taiwan, GengZiPeng | KC313271 |
| | clone12.ENVS.GZP.epilithic.low11 | Taiwan, GengZiPeng | KC313272 |
| | clone12.ENVS.GZP.epilithic.low12 | Taiwan, GengZiPeng | KC313273 |
| | clone12.ENVS.GZP.epilithic.low13 | Taiwan, GengZiPeng | KC313274 |
| | clone12.ENVS.GZP.epilithic.low14 | Taiwan, GengZiPeng | KC313275 |
| | clone12.ENVS.GZP.epilithic.low15 | Taiwan, GengZiPeng | KC313276 |
| | clone12.ENVS.GZP.epilithic.low16 | Taiwan, GengZiPeng | KC313277 |
| | clone12.ENVS.GZP.epilithic.low18 | Taiwan, GengZiPeng | KC313278 |
| | clone12.ENVS.GZP.epilithic.low19 | Taiwan, GengZiPeng | KC313279 |
| | clone12.ENVS.GZP.epilithic.low20 | Taiwan, GengZiPeng | KC313280 |
| | clone12.ENVS.GZP.epilithic.low21 | Taiwan, GengZiPeng | KC313281 |
| | clone12.ENVS.GZP.epilithic.low22 | Taiwan, GengZiPeng | KC313282 |
| | clone12.ENVS.GZP.epilithic.low23 | Taiwan, GengZiPeng | KC313283 |
| | clone12.ENVS.GZP.epilithic.low24 | Taiwan, GengZiPeng | KC313284 |
| | clone12.ENVS.GZP.epilithic.low25 | Taiwan, GengZiPeng | KC313285 |
| | clone12.ENVS.GZP.epilithic.low26 | Taiwan, GengZiPeng | KC313286 |
| | clone12.ENVS.GZP.epilithic.low27 | Taiwan, GengZiPeng | KC313287 |
| | clone12.ENVS.GZP.epilithic.low28 | Taiwan, GengZiPeng | KC313288 |
| | clone12.ENVS.GZP.epilithic.low29 | Taiwan, GengZiPeng | KC313289 |
| | clone12.ENVS.GZP.epilithic.low30 | Taiwan, GengZiPeng | KC313290 |
| | clone12.ENVS.GZP.soil.low1 | Taiwan, GengZiPeng | KC313291 |
| | clone12.ENVS.GZP.soil.low3 | Taiwan, GengZiPeng | KC313292 |
| | clone12.ENVS.GZP.soil.low4 | Taiwan, GengZiPeng | KC313293 |
| | clone12.ENVS.GZP.soil.low5 | Taiwan, GengZiPeng | KC313294 |
| | clone12.ENVS.GZP.soil.low9 | Taiwan, GengZiPeng | KC313295 |
| | clone12.ENVS.GZP.soil.low11 | Taiwan, GengZiPeng | KC313296 |
| | clone12.ENVS.GZP.soil.low12 | Taiwan, GengZiPeng | KC313297 |
| | clone12.ENVS.GZP.soil.low13 | Taiwan, GengZiPeng | KC313298 |
| | clone12.ENVS.GZP.soil.low17 | Taiwan, GengZiPeng | KC313299 |
| | clone12.ENVS.GZP.stream45.1.1 | Taiwan, GengZiPeng | JX981609 |
| | clone12.ENVS.GZP.stream45.1.4 | Taiwan, GengZiPeng | JX981611 |
| | clone12.ENVS.GZP.stream45.1.6 | Taiwan, GengZiPeng | JX981612 |
| | clone12.ENVS.GZP.stream45.1.7 | Taiwan, GengZiPeng | JX981613 |
| | clone12.ENVS.GZP.stream45.1.8 | Taiwan, GengZiPeng | JX981614 |
| | clone12.ENVS.GZP.stream45.1.9 | Taiwan, GengZiPeng | JX981615 |
| | clone12.ENVS.GZP.stream45.1.10 | Taiwan, GengZiPeng | JX981616 |
| | clone12.ENVS.GZP.stream45.1.11 | Taiwan, GengZiPeng | JX981617 |
| | clone12.ENVS.GZP.stream45.1.12 | Taiwan, GengZiPeng | JX981618 |
| | clone12.ENVS.GZP.stream45.1.13 | Taiwan, GengZiPeng | JX981619 |
| | clone12.ENVS.GZP.stream45.1.16 | Taiwan, GengZiPeng | JX981620 |
| | clone12.ENVS.GZP.stream45.1.20 | Taiwan, GengZiPeng | JX981621 |
| | clone12.ENVS.GZP.stream45.1.21 | Taiwan, GengZiPeng | JX981622 |
| | clone12.ENVS.GZP.stream45.1.22 | Taiwan, GengZiPeng | JX981623 |
| | clone05.ENVS.DRG.stream42.sun.1 | Taiwan, GengZiPeng | JX981642 |
| | clone12.ENVS.MC.sulfurFume1.1.2 | Taiwan, MaChao | JX981516 |
| | clone12.ENVS.MC.sulfurFume1.1.6 | Taiwan, MaChao | JX981517 |
| | clone12.ENVS.MC.sulfurFume1.1.7 | Taiwan, MaChao | JX981518 |
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| | clone12.ENVS.MC.sulfurFume1.2.3 | Taiwan, MaChao | JX981519 |
| | clone12.ENVS.MC.sulfurFume1.1.3 | Taiwan, MaChao | JX981521 |
| | clone12.ENVS.MC.sulfurFume1.3.5 | Taiwan, MaChao | JX981523 |
| | clone12.ENVS.MC.sulfurFume1.3.7 | Taiwan, MaChao | JX981524 |
| | clone12.ENVS.MC.sulfurFume1.3.8 | Taiwan, MaChao | JX981525 |
| | clone12.ENVS.MC.sulfurFume1.3.16 | Taiwan, MaChao | JX981528 |

| Taxa | Strain | Sampling Site | GenBank number |
|---|---|---|---|
| ***Cyanidium caldarium*** | ACUF767 | Turkey, Cermik | KY033432 |
| | ACUF775 | Turkey, Diyadin | KY033437 |
| | CloneT17 | Turkey, Güçlükonak | KY033462 |
| ***Cyanidioschyzon merolae*** | CloneT01 | Turkey, Biloris | KY033448 |
| | CloneT05 | Turkey, Nemrut | KY033452 |
| ***Galdieria maxima*** | ACUF653 | Turkey,Biloris | KY033400 |
| | ACUF764 | Turkey,Biloris | KY033430 |
| | ACUF763 | Turkey,Biloris | KY033429 |
| | ACUF735 | Turkey,Biloris | KY033422 |
| | ACUF698 | Turkey,Biloris | KY033416 |
| | ACUF650 | Turkey, Cermik | KY033398 |
| | CloneT03 | Turkey, Cermik | KY033450 |
| | ACUF647 | Turkey, Cermik | KY033396 |
| | cloneT04 | Turkey, Cermik | KY033451 |
| | ACUF766 | Turkey, Cermik | KY033431 |
| | ACUF783 | Turkey, Cermik | KY033443 |
| | ACUF774 | Turkey,Diyadin | KY033436 |
| | CloneT06 | Turkey,Diyadin | KY033453 |
| | ACUF665 | Turkey,Diyadin | KY033406 |
| | CloneT13 | Turkey,Diyadin | KY033460 |
| | ACUF772 | Turkey,Diyadin | KY033435 |
| | cloneT18 | Turkey,Diyadin | KX501185 |
| | ACUF773 | Turkey,Diyadin | KX501180 |
| | ACUF671 | Turkey,Manisa Kula | KY033410 |
| | ACUF648 | Turkey,Manisa Kula | KY033397 |
| | ACUF731 | Turkey,Manisa Kula | KY033420 |
| | cloneT12 | Turkey,Manisa Kula | KY033459 |
| | ACUF776 | Turkey,Manisa Kula | KY033438 |
| | ACUF777 | Turkey,Manisa Kula | KY033439 |
| | CloneT14 | Turkey,Manisa Kula | KY033461 |
| | ACUF743 | Turkey,Manisa Kula | KY033428 |
| | ACUF741 | Turkey,Manisa Kula | KY033426 |
| | ACUF782 | Turkey,Manisa Kula | KY033442 |
| | ACUF673 | Turkey,Germencik | KY033411 |
| | ACUF739 | Turkey,Germencik | KY033425 |
| | ACUF736 | Turkey,Germencik | KY033423 |
| | CloneT15 | Turkey,Germencik | KX501183 |
| | ACUF742 | Turkey,Germencik | KY033427 |
| | ACUF660 | Turkey, Güçlükonak | KY033404 |
| | ACUF697 | Turkey, Güçlükonak | KY033415 |
| | ACUF722 | Turkey, Güçlükonak | KX501174 |
| | ACUF769 | Turkey, Güçlükonak | KX501179 |
| | ACUF724 | Turkey, Güçlükonak | KY033419 |
| | ACUF714 | Turkey, Güçlükonak | KY033418 |
| | ACUF695 | Turkey, Güçlükonak | KY033414 |
| | ACUF710 | Turkey, Güçlükonak | KX501173 |
| ***Galdieria phlegrea*** | ACUF657 | Turkey,Biloris | KY033402 |
| | ACUF656 | Turkey,Biloris | KY033401 |
| | ACUF652 | Turkey,Biloris | KY033399 |
| | ACUF780 | Turkey,Biloris | KX501182 |
| | ACUF765 | Turkey,Biloris | KX501177 |
| | ACUF625 | Turkey, Cermik | KY033394 |
| | ACUF668 | Turkey, Cermik | KY033408 |
| | CloneT07 | Turkey, Cermik | KY033454 |
| | ACUF642 | Turkey, Cermik | KY033395 |
| | CloneT08 | Turkey, Cermik | KY033455 |
| | CloneT10 | Turkey, Cermik | KY033457 |
| | ACUF667 | Turkey,Diyadin | KY033407 |
| | ACUF669 | Turkey,Diyadin | KY033409 |
| | ACUF 771 | Turkey,Diyadin | KY033434 |
| | ACUF 658 | Turkey,Guklukonak | KY033403 |
| | ACUF737 | Turkey,Diyadin | KY033424 |
| | ACUF 734 | Turkey,Diyadin | KY033421 |
| | ACUF 787 | Turkey,Diyadin | KY033446 |
| | ACUF785 | Turkey,Diyadin | KY033445 |
| | cloneT09 | Turkey,Gucklukonak | KY033456 |
| | ACUF784 | Turkey,Gucklukonak | KY033444 |
| | ACUF770 | Turkey,Gucklukonak | KY033433 |
| | cloneT16 | Turkey,Gucklukonak | KX501184 |
| | cloneT11 | Turkey,Manisa Kula | KY033458 |
| | ACUF664 | Turkey,Nemrut | KY033405 |

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|---|---|---|---|
| | ACUF738 | Turkey,Nemrut | KX501176 |
| | ACUF788 | Turkey,Dyadin | KY033447 |
| ***Galdieria sulphuraria*** | ACUF779 | Turkey,Germencik | KY033440 |
| | ACUF676 | Turkey,Germencik | KY033413 |
| | cloneT02 | Turkey,Germencik | KY033449 |
| | ACUF674 | Turkey,Germencik | KY033412 |
| | ACUF778 | Turkey,Germencik | KX501181 |
| | ACUF781 | Turkey,Gucklukonak | KY033441 |
| | ACUF725 | Turkey,Gucklukonak | KX501175 |
| | ACUF768 | Turkey,Gucklukonak | KX501178 |
| | ACUF700 | Turkey,Gucklukonak | KY033417 |

**Table S1.** GenBank Accession numbers for taxa included in the phylogenetic analyses.


Agri-Diyadin
Kula-Manisa
Nemrut-Bitlis
Germencik-Aydin
Cermik-Dyiarbakir
Biloris-Siirt
Sirnak